Practitioner's Docket No.: 789_070 *PATENT*

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the application of: Toshikazu HIROTA and Takao OHNISHI

Ser. No.: 09/868,832 Group Art Unit: 1634

Filed: June 21, 2001 Examiner: Forman, B.

Confirmation No.: 6274

For: BIOCHIP

RULE 132 DECLARATION

I, Toshikazu Hirota, hereby declare and state that:

1. I am one of the inventors named in the above-identified patent application.

2. I received a BS Degree in Chemistry from Kyoto University in 1985 and a Masters Degree in Chemistry from Kyoto University in 1987. I have been employed by NGK Insulators, Ltd., the assignee of the present application, since April of 1987. During my employment at NGK Insulators, Ltd., I have been involved in research and development of electronic ceramics and, since 1992, particularly in the area of piezoelectric/electrostrictive devices.

3. I am familiar with the prosecution history of the above-identified application, and have reviewed the Office Action mailed July 28, 2003. It is my understanding that the PTO Examiner believes that biochips formed using an ink-jet apparatus, as claimed, are not structurally distinct from biochips formed by pin-head arraying devices. The present invention, however, is based, at least in part, on the discovery that the ink-jet process provides a structurally distinct biochip over biochips formed using conventional pin-head methods.

4. The ink-jet process, as compared to conventional pin-head methods, provides a structurally distinct biochip, because of the non-contact nature of the ink-jet process. In accordance with the invention, a first ink-jet sample spot that has a desired spot size and/or a desired capture material concentration is supplied onto a base plate and allowed to dry. A second ink-jet sample spot, which, for example, can have the same or a different spot size in relation to the first ink-jet sample spot and/or the same or a different capture material concentration, is then supplied on top of the dried first ink-jet sample spot. Due to the non-contact nature of the ink-jet process, the integrity of the dried first ink-jet sample spot is not affected by the delivery of the second ink-jet supplied sample spot. This is not the case with pin-head methods, in that the pin must contact the base plate during every application of material. This contact adversely affects the integrity of any spots already present on the base plate and thus adversely affects the uniformity of spot concentration across the test slide.

5. The following experiments were conducted by me or under my supervision to prove that use of an ink-jet process, as claimed, precisely delivers capture solution spots having a more accurate and reproducible capture material concentration in comparison to capture solution spots that are supplied onto a base plate using conventional pin-head methods.

6. The Table attached hereto shows the results of a direct comparison between the variations of fluorescent signal intensities emitted from ink-jet delivered capture solution spots having a relative capture material concentration (i.e., target concentration of capture solution 1, 2 or 3) and capture solution spots having the same relative capture material concentrations delivered by a pin-head method. A total of 60 spots were formed on a base plate. Each spot within the first group of 30 spots was formed using the ink-jet method, while each spot in the second group of 30 spots was formed using the pin-head method. After the first drops were allowed to dry, a second sample drop was supplied using the ink-jet method to 20 of the 30 spots in the first group,

and a second sample drop was supplied using the pin-head method to 20 of the 30 spots in the second group. After the second drops were allowed to dry, a third sample drop was supplied using the ink-jet method to 10 of the two-drop spots in the first group, and a third drop was supplied using the pin-head method to 10 of the two-drop spots in the second group. The finished test slide, therefore, had two groups of 30 spots, and within each group there were 10 one-drop spots, 10 two-drop spots, and 10 three-drop spots.

7. A solution including a target material capable of reacting with the corresponding capture materials in the capture solution spots formed on the base plate was then supplied onto each spot. The capture solutions used to form the spots contained relative concentrations of capture materials (i.e., target concentration of capture material 1, 2 or 3) that were adapted to specifically react with the target materials in the target material solution which was, for example, a positive control. The reaction between the capture solution spots and the target material solution caused a fluorescent signal to be emitted from each reacted capture solution spot. The fluorescent signal intensity value from each spot was obtained by subtracting background fluorescence, such as, for example, fluorescence emitted from the base plate itself, from the total fluorescence emanating from the reacted capture solution spots and the base plate. The capture solution fluorescent signal intensities were detected and measured using a conventional scanning apparatus.

8. The Table shows that the fluorescent signal intensities emitted from each of the individual spots within each capture spot group of pin-head delivered capture solution spots vary significantly from one another in comparison to the much smaller variation between the signal intensities emitted from the individual spots within each capture spot group of ink-jet supplied capture solution spots. For example, while the standard deviation of signal intensities between ink-jet delivered spots in the relative target concentration capture spot groups 1-3 was 310, 311 and 435, respectively, the standard deviation of signal intensities between pin-head delivered spots in the same

relative target concentration capture spot groups 1-3 was 656, 889 and 979, respectively. The lower standard deviation between the ink-jet delivered capture solution spots, as compared to the higher standard deviation between the pin-head delivered capture solution spots, proves that the claimed ink-jet process consistently delivers capture solution spots having a more exact, desired amount of capture material per sample spot. This is due to the non-contact nature of the ink-jet method.

9. The relatively higher standard deviation between the pin-head delivered sample spots is due to the contact, disruptive nature of the pin-head method. These results show that the degree of accuracy and precision in capture material per spot attributable to the claimed ink-jet process is not attainable using prior art pin-head arraying devices.

10. The comparative table of signals also shows that a significant variation exists between the standard deviation of fluorescent signal intensities emitted from each group of capture solution spots delivered using a pin-head device when compared to the signal intensities of those same groups of capture solution spots supplied onto the base plate via the ink-jet process. For example, while the standard deviation values for the signal intensities between the ink-jet delivered spots in relative target concentration capture spot groups 1 and 2 are practically identical (i.e, the standard deviations are 310 and 311, respectively), a much wider gap exists between the standard deviation values for the signal intensities between the pin-head delivered spots in relative target concentration capture spot groups 1 and 2 (i.e, the standard deviations are 656 and 889, respectively).

11. The practically identical standard deviation values for the signal intensities emitted from the ink-jet delivered capture solution spots in relative target concentration capture spot groups 1 and 2 is attributable to the non-contact nature of the ink-jet process. That is, the dried first ink-jet sample spot (having a target concentration of 1) is not affected by the delivery of the second ink-jet supplied sample spot (to provide an overall target concentration of 2). The standard deviation values for the signal intensities

between the pin-head delivered spots in relative target concentration capture spot groups 1 and 2 varies widely due to the contact nature of the pin-head method. These results show that the more exact, desired amount of capture material per sample spot attributable to the claimed ink-jet process is not attainable using prior art pin-head arraying devices.

12. The graph attached hereto provides additional quantitative proof that the non-contact nature of the claimed ink-jet process makes it possible to reproducibly deliver a more exact, desired amount of capture material per sample spot as compared to prior art pin-contact methods. The graph shows the existence of a linear relationship between the emitted signal intensities (which correspond to the relative target concentrations of each of the sample spots) of each of the ink-jet capture spot groups 1-3. That is, there is a clear linear relationship between the 3 groups. While the signal intensity values for ink-jet capture spot groups 1-3 are tightly packed around their respective average signal intensity values, it is difficult to ascertain whether the emitted signal intensity values for each of the pin-head capture spot groups 1-3 even belong to the same capture spot group. For example, it is clear that the signal intensities for the pin-head capture spot groups 1-3 are widely scattered and there are several instances in which the signal intensity values for one capture spot group overlap with the signal intensity values of another capture spot group. Accordingly, the graph results provide further proof that the more exact, desired and reproducible amount of capture material per sample spot attributable to the claimed ink-jet process is not attainable using prior art pin-contact arraying devices.

13. The results of this experimentation clearly show that the claimed ink-jet process provides a structurally distinct biochip in comparison to a biochip that is fabricated using conventional pin-head methods.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

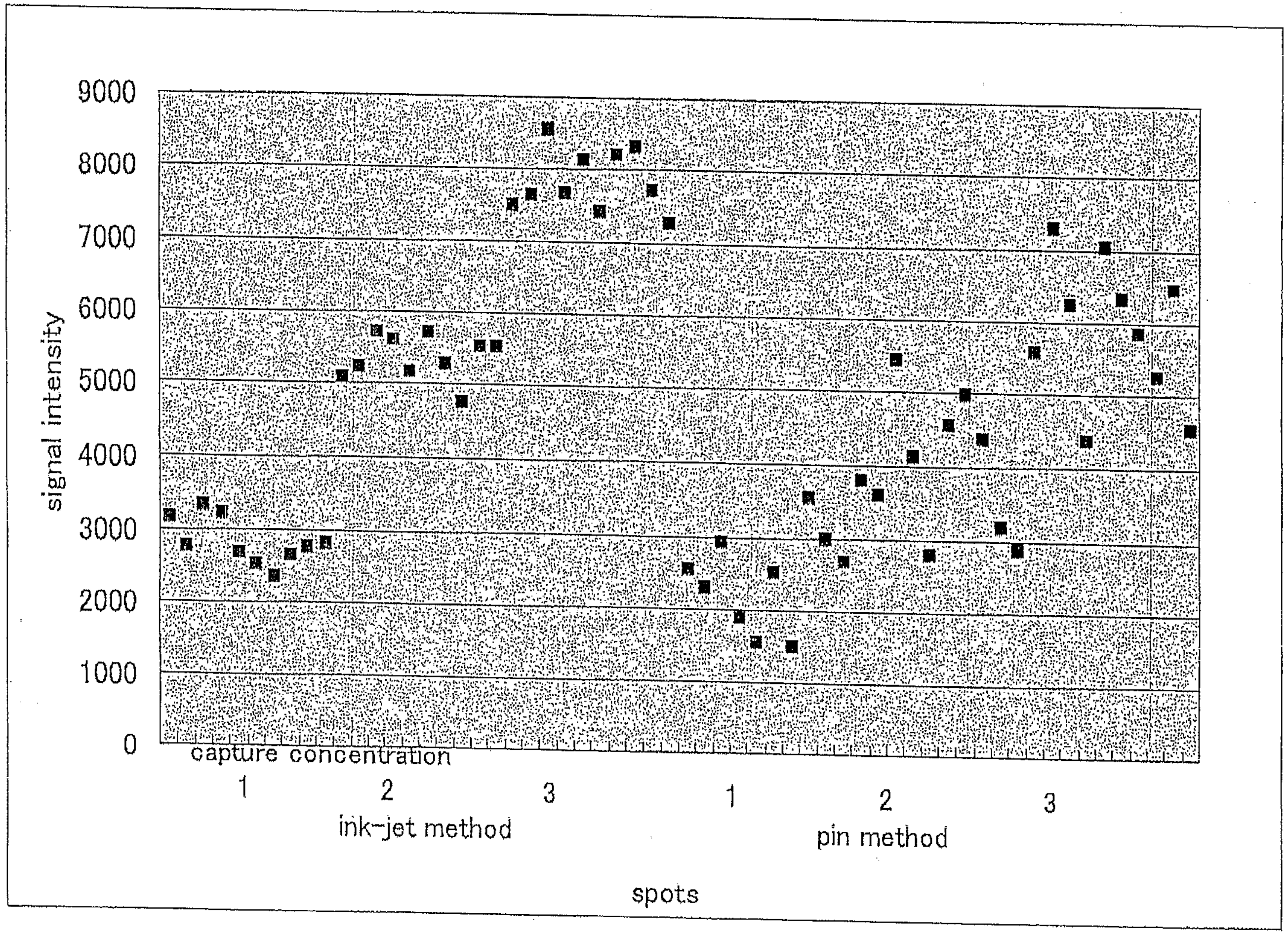
December 18, 2003
Date

Toshikazu Hirota
Toshikazu Hirota

Enclosures:
Comparative Table of Signals
Comparative Graph of Signal Intensities

Comparative table of signals which are obtained ink-jet method and pin method

spotting method	target concentratio of capture	measured value spot	background	signal intensity	average	standard deviation	(%) cofficient of variati
ink-jet method	1	3752	583	3169			
	1	3345	576	2769			
	1	3882	569	3313			
	1	3783	575	3208			
	1	3264	576	2688			
	1	3090	577	2513			
	1	2958	598	2360			
	1	3256	585	2671			
	1	3345	578	2767			
	1	3392	575	2817	2828	310	10.97
	2	5656	573	5083			
	2	5826	580	5246			
	2	6312	582	5730			
	2	6187	576	5611			
	2	5755	574	5181			
	2	6311	581	5730			
	2	5871	570	5301			
	2	5359	585	4774			
	2	6123	574	5549			
	2	6107	571	5536	5374	311	5.79
	3	8084	579	7505			
	3	8222	589	7633			
	3	9142	587	8555			
	3	8246	582	7664			
	3	8720	578	8142			
	3	8030	610	7420			
	3	8805	583	8222			
	3	8928	580	8348			
	3	8310	582	7728			
	3	7847	576	7271	7849	435	5.54
pin method	1	3129	579	2550			
	1	2865	572	2293			
	1	3507	576	2931			
	1	2467	575	1892			
	1	2135	587	1548			
	1	3083	560	2523			
	1	2076	579	1497			
	1	4135	580	3555			
	1	3567	579	2988			
	1	3257	574	2683	2446	656	26.81
	2	4373	574	3799			
	2	4179	568	3611			
	2	6017	564	5453			
	2	4683	566	4117			
	2	3377	580	2797			
	2	5116	551	4565			
	2	5546	562	4984			
	2	4939	567	4372			
	2	3745	568	3177			
	2	3442	560	2882	3976	889	22.36
	3	6153	553	5600			
	3	7856	555	7301			
	3	6819	554	6265			
	3	4921	551	4370			
	3	7598	550	7048			
	3	6889	549	6340			
	3	6414	550	5864			
	3	5797	549	5248			
	3	7040	550	6490			
	3	5102	551	4551	5908	979	16.58



signal intensity
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
capture concentration
1
2
3
ink-jet method
1
2
3
pin method
spots